

Fabrication and evaluation of enzyme loaded microgel shapes as carriers for intravenous drug delivery

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In the current work, successful fabrication of biocompatible rod-like microgel shapes as carriers intended for intravenous drug delivery is demonstrated. The microgel shapes with a length of 8 μm and a width and height of 2 μm are fabricated by UV-assisted punching. UV-assisted punching is an up-scalable technique for microgel shape development across the size range of 8-100 μm . Hemolysis assays with the microgel shapes indicate their hemocompatibility. The microgel shapes could be loaded with model enzyme β -lactamase and an enzymatic response could be obtained from them.

Hydrogels are 3D polymeric matrices that absorb water while maintaining their structural integrity when placed in aqueous environments. They have become an increasingly important class of drug delivery carriers due to their biocompatibility, porous matrix and high water content, making them akin to biological cells and tissue. [1] Thus, hydrogels are particularly interesting for the encapsulation of sensitive cargo such as proteins and enzymes. Traditionally, micro- and nano-hydrogels are fabricated by bottom-up techniques, yielding polydisperse spherical carriers. [2] However, recent studies highlight that carrier shape and size play a significant role in carrier flow, internalization and interaction with biological membranes. [3] As a result, there has been high impetus in developing monodisperse and shape specific carriers for drug delivery, bringing top-down techniques to the forefront for their development.

Recently, we have introduced UV-assisted punching as a novel technique for the top-down fabrication of microgel shapes with varying geometries. First, the geometry of the microgel shapes is defined in a Si master, fabricated by photolithography and reactive ion-etching (RIE). This master is then used to transfer the inverse geometry into a cyclo-olefin polymer (COP) foil by hot embossing. The wells of the stamp are loaded with the hydrogel precursor by force assisted liquid distribution (FALD) on a roll-to-plate imprinter (Fig. 1A-C). Thereafter, the loaded stamp was assembled with a poly-vinyl alcohol (PVA) substrate and UV-assisted punching was performed to obtain individual microgel shapes (Fig. 1D-H). In this process, the hydrogel precursor was crosslinked by UV radiation while the stamp was pressed into the PVA substrate, penetrating it to define individual microgel shapes. The assembly is manually demolded to obtain the punched microgel shapes on the PVA substrate.

The fabricated rod-like structures in Si, the corresponding wells in a COP stamp and finally the microgel shapes on PVA are shown in Fig 2. The fabricated microgel shapes were harvested in a phosphate buffered saline (PBS) solution, washed and filtered. The hemocompatibility of the resultant microgel shapes was evaluated with blood cells to demonstrate their potential as carriers for intravenous drug delivery. A 2% hemolysis rate of the blood cells is observed with microgel shape concentrations up to 8,000,000 (Fig. 3A). With a hemolysis rate well below the usual threshold of 5%, it can be concluded that the rod-like microgel shapes are hemocompatible. [4] Furthermore, microscopic evaluation of the cells also shows that they maintain their native shape post incubation with microgel shapes at various concentrations. (Fig. 3B).

The biocompatibility of the microgel shapes is currently being evaluated through cell viability assays and their loading and response with bioactive enzyme β -lactamase is explored.

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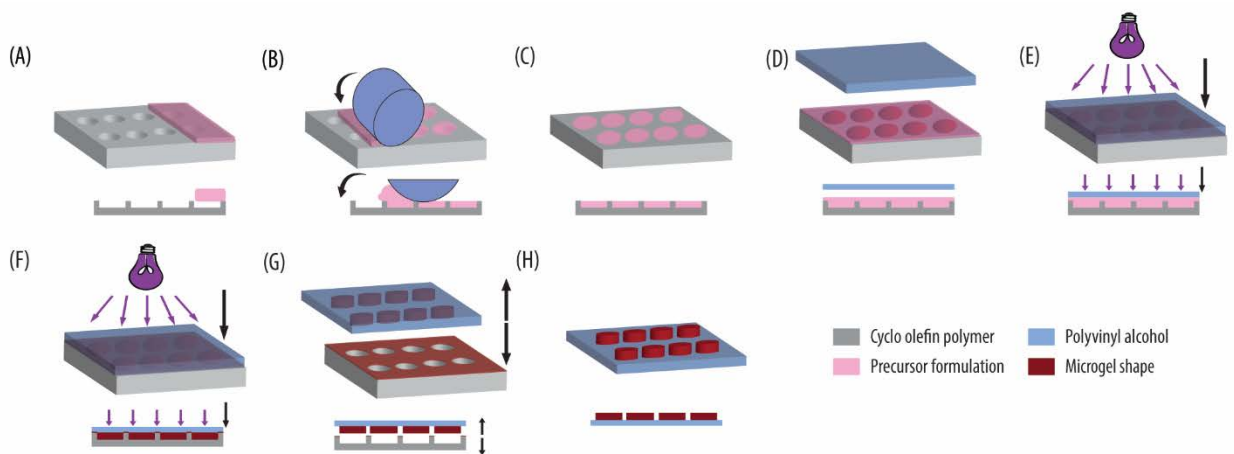


Figure 1. Illustration of (A)-(C) force assisted liquid distribution (FALD) to load the stamp wells and (D)-(H) UV-assisted punching to fabricated individual microgel shapes. FALD: (A) Hydrogel precursor is cast on a COP stamp, (B) the deposited liquid is pushed into the wells of the stamp sequentially by a force via a R2P embosser, (C) a stamp fully loaded with the hydrogel precursor. UV-assisted punching: (D) the loaded stamp is assembled with the PVA substrate, (E) irradiation of the assembly while applying a pressure (F) crosslinking of the loaded hydrogel precursor due to the irradiation while the applied pressure cuts through the hydrogel precursor flash layer and penetrating the PVA to form microgel shapes, (G) manual demolding of the PVA substrate from the COP stamp and, (H) Individual microgel shapes on the PVA.

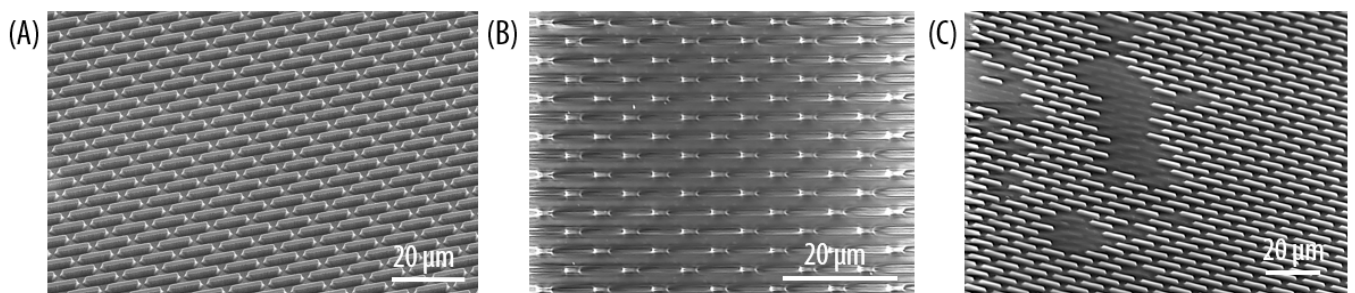


Figure 2. SEM micrographs of 8 X 2 X 2 μm rod-like: (A) structures in Si fabricated by photolithography and reactive ion etching (RIE), (B) wells hot embossed into a COP foil with the Si master and, (C) microgel shapes on a PVA substrate fabricated with the COP stamp by force assisted liquid distribution (FALD) and UV-assisted punching

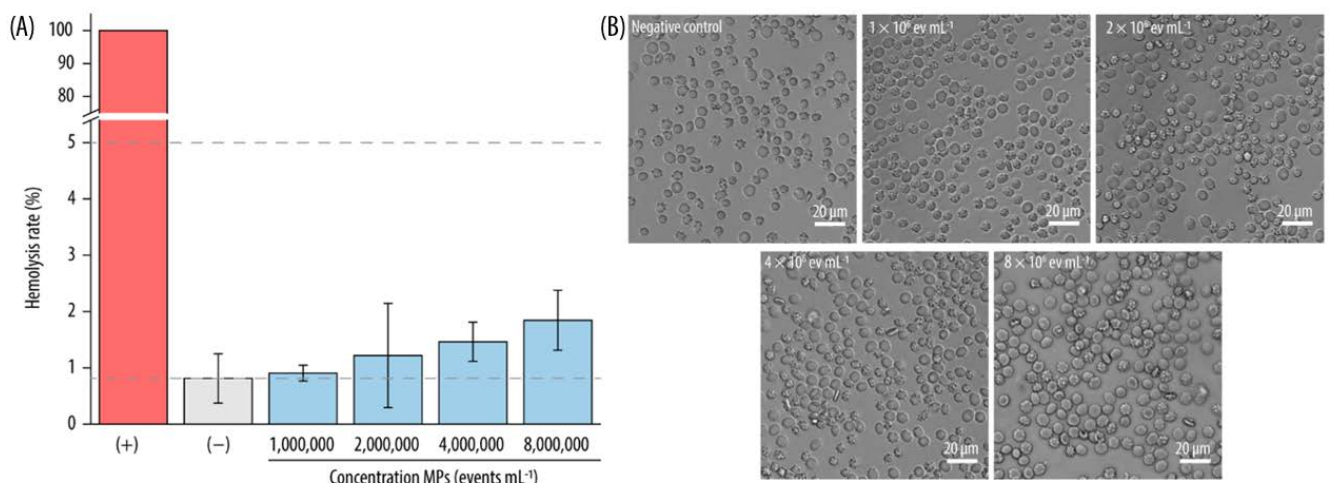


Figure 3. (A) Hemolysis rate of blood cells during after incubation with various concentrations of rod-like microgel shapes and, (B) Bright field microscopic images of blood cells post incubation with rod-like microgel shapes.